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16. ABSTRACT

Samples of commercial feeds for laboratory rats, guinea pigs, cats, monkeys,rabbits, and hamsters were collected and analyzed qualitatively and quantitatively for selected antibiotics, trace metals, pesticides (organophosphates and chlorinated hydrocarbons), natural agents, and polychlorinated biphenyls (PCBs). The results indicated that antibiotics, parathion, diazinon, aldrin, and the aflatoxins were not detected, whereas, metals, chlorinated hydrocarbon pesticides (except aldrin), and PCBs were continually present. Malathion and estrogen were found occasionally.

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Contaminant Levels in Animal Feeds Used for Toxicity Studies

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Abstract. Samples of commercial feeds for laboratory rats, guinea pigs, cats, monkeys, rabbits, and hamsters were collected and analyzed qualitatively and quantitatively for selected antibiotics, trace metals, pesticides (organophosphates and chlorinated hydrocarbons), natural agents, and polychlorinated biphenyls (PCBs). The results indicated that anitibiotics, parathion, diazinon, aldrin, and the aflatoxins were not detected, whereas, metals, chlorinated hydrocarbon pesticides (except aldrin), and PCBs were continually present. Malathion and estrogen were found occasionally.

An advantage of toxicity studies employing laboratory animals is the ability to control the environment and thus determine with more precision and accuracy the adverse effects of agents under investigation. Recognition of the ubiquitous nature of many environmental contaminants in the human food supply warrants close scrutiny of the feeds of experimental animals for levels of contaminants that may modify the interpretation of toxicity studies, particularly those of relatively long duration (e.g., subchronic, reproductive, and chronic toxicity studies). Our laboratory has undertaken a continuous quality assurance program aimed at characterization and quantitation of selected feed contaminants. This report describes the results of analyses of Purina® feeds collected over a consecutive 12-month period. The contaminants detected and their levels are pertinent only to this period of time and to the diets specified in the Tables. The listings of contaminants are not to be considered all inclusive. Also, just because a contaminant, such as lindane or the phthalate esters, is not mentioned does not mean this contaminant presents no problem.

Materials and Methods

Qualitative and quantitative analyses for selected contaminants in every "lot" of animal feed received by this Laboratory were performed on a monthly basis. Three kilograms of each animal diet provided enough feed for duplicate analyses of all contaminants.

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Table 1. Trace contaminants analyzed in experimental animal feeds

Compound	Limit of detection	Analytical[a] reference
Antibiotics (ppm)		AOAC 1975[b]
Chlortetracycline	0.55	
Oxytetracycline	1.21	
Penicillin	2.2	
Streptomycin	2.85	
Aflatoxins (ppb) (B_1, B_2, G_1, G_2)	2	Pons *et al.* 1970
Estrogens (ppb)	4	NCTR 1973[c]
Polychlorinated biphenyls (PCBs) (ppb)	10	AOAC 1975
Pesticides (ppb) Aldrin, dieldrin, DDD, DDE, DDT, endrin, heptachlor, heptachlor epoxide, malathion, parathion, and diazinon	5	AOAC 1975
Metals (ppm)		AOAC 1975
Arsenic	0.05	
Cadmium	0.05	
Lead	0.05	
Manganese	0.01	
Mercury		
Digestion		Analytical Methods Committee 1961
Determination	0.05	Hatch and Ott 1968
Selenium	0.05	Gutenmann and Lisk 1961

[a] The specific references for methods listed here have been used routinely by the contractor for years. Their experience with replicate and recovery work indicated that certain methods were applicable to a wide variety of matrices

[b] Official Methods of Analysis, Association of Official Analytical Chemists

[c] National Center for Toxicological Research

Table 1 lists the compounds analyzed in feed samples. Methods of analysis are referenced and the limits of detection noted.

Results

Rat Diet

Results of analyses of Purina Rat Diet, collected over a one-year period, are presented in Table 2. The following were not detected in any of the feed samples: Antibiotics, parathion, diazinon, aflatoxins, and aldrin. By contrast, trace

Table 2. Results of analyses of rat diet for trace contaminants

Substance	Samples detected / samples analyzed	Median (N)	Mean (N)	Range
Antibiotics (ppm)				
Penicillin	0/12	—	—	—
Streptomycin	0/12	—	—	—
Oxytetracycline	0/12	—	—	—
Chlortetracycline	0/12	—	—	—
Metals (ppm)				
Arsenic	12/12	0.39 (12)	0.41 (12)	0.32 – 0.51
Cadmium	12/12	0.10 (12)	0.11 (12)	0.08 – 0.15
Lead	12/12	0.42 (12)	0.50 (12)	0.25 – 1.68
Manganese	12/12	65.4 (12)	69.7 (12)	39.8 – 112.6
Mercury	12/12	0.05 (12)	0.05 (12)	—
Selenium	12/12	0.34 (12)	0.34 (12)	0.21 – 0.46
Pesticides				
Organophosphates (ppm):				
Malathion	9/12	0.09 (9)	0.09 (9)	0.04 – 0.13
Parathion	0/12	—	—	—
Diazinon	0/12	—	—	—
Chlorinated hydrocarbons (ppb):				
DDT	11/11	5 (11)	15 (5)	5 – 24
DDE	11/11	5 (11)	13 (5)	5 – 22
DDD	11/11	5 (11)	8 (4)	5 – 13
Aldrin	0/11	—	—	—
Dieldrin	11/11	5 (11)	5 (11)	—
Endrin	11/11	5 (11)	5 (11)	—
Heptachlor epoxide	11/11	5 (11)	5 (11)	—
Natural compounds (ppb)				
Aflatoxin B-1	0/12	—	—	—
B-2	0/12	—	—	—
G-1	0/12	—	—	—
G-2	0/12	—	—	—
Estrogen (ppb)	7/12	4 (7)	4 (7)	—
Miscellaneous				
Polychlorinated biphenyls (PCBs) (ppb)	11/12	17 (11)	39 (11)	10 – 77

elements and chlorinated hydrocarbon pesticides (with the exception of aldrin) were detected in all samples of feed analyzed. Malathion was detected in only 9 samples, estrogen in only 7 of the analyzed samples, and PCBs in 11 of 12 samples. Of the trace elements, the concentrations of manganese were several orders of magnitude greater than the concentrations of the other trace elements. While detectable, the levels of mercury were not quantifiable. Where the levels were quantifiable, the ranges indicate that the concentrations measured were within relatively narrow limits.

Guinea Pig Diet

Purina Guinea Pig Diet was collected over a one-year period and samples were analyzed for various contaminants. The results are listed in Table 3. The fol-

Table 3. Results of analyses of guinea pig diet for trace contaminants

Substance	Samples detected / samples analyzed	Median (N)	Mean (N)	Range
Antibiotics (ppm)				
Penicillin	0/12	—	—	—
Streptomycin	0/12	—	—	—
Oxytetracycline	0/12	—	—	—
Chlortetracycline	0/12	—	—	—
Metals (ppm)				
Arsenic	12/12	0.21 (12)	0.21 (12)	0.12 – 0.40
Cadmium	12/12	0.14 (12)	0.14 (12)	0.10 – 0.20
Lead	12/12	0.40 (12)	0.44 (12)	0.27 – 0.73
Manganese	12/12	69.0 (12)	70.4 (12)	59.9 – 78.3
Mercury	12/12	0.05 (12)	0.05 (12)	—
Selenium	12/12	0.22 (12)	0.26 (12)	0.18 – 0.40
Pesticides				
Organophosphates (ppm):				
Malathion	9/12	0.90 (9)	0.17 (9)	0.05 – 0.40
Parathion	0/12	—	—	—
Diazinon	0/12	—	—	—
Chlorinated hydrocarbons (ppb):				
DDT	11/11	5 (11)	5 (11)	—
DDE	11/11	5 (11)	5 (11)	—
DDD	11/11	5 (11)	5 (11)	—
Aldrin	0/11	—	—	—
Dieldrin	11/11	5 (11)	5 (11)	—
Endrin	11/11	5 (11)	5 (11)	—
Heptachlor epoxide	11/11	5 (11)	5 (11)	—
Natural compounds (ppb)				
Aflatoxin B-1	0/12	—	—	—
B-2	0/12	—	—	—
G-1	0/12	—	—	—
G-2	0/12	—	—	—
Estrogen (ppb)	8/12	4 (8)	4 (8)	—
Miscellaneous				
Polychlorinated biphenyls (PCBs) (ppb)	11/12	10 (11)	11 (2)	10 – 11

lowing contaminants were not detected in any samples analyzed: Antibiotics, parathion, diazinon, the aflatoxins, and aldrin. The following were detected in all samples analyzed: Trace metals, DDT and its metabolites, dieldrin, endrin, and heptachlor epoxide. Malathion, estrogen, and PCBs were found in 9, 8, and 11 of the samples analyzed, respectively. With the exception of manganese, all of the trace contaminants were in the part per billion range. By contrast, manganese was several orders of magnitude greater in concentration than the other trace contaminants. As noted in the range of concentrations for the individual contaminants, occurrence of the individual agents was within relatively narrow limits throughout the year.

Cat Diet

Over a one-year period, samples were collected of Purina Cat Diet and analyzed for various trace elements. The results of these analyses are presented

Table 4. Results of analyses of cat diet for trace contaminants

Substance	Samples detected / samples analyzed	Median (N)	Mean (N)	Range
Antibiotics (ppm)				
Penicillin	0/12	—	—	—
Streptomycin	0/12	—	—	—
Oxytetracycline	0/12	—	—	—
Chlortetracycline	1/12	—	—	—
Metals (ppm)				
Arsenic	12/12	0.32 (12)	0.47 (12)	0.24 – 0.70
Cadmium	12/12	0.12 (12)	0.13 (12)	0.09 – 0.19
Lead	12/12	1.04 (12)	0.95 (12)	0.34 – 1.55
Manganese	12/12	66.1 (12)	66.7 (12)	60.7 – 75.4
Mercury	12/12	0.05 (12)	0.05 (12)	—
Selenium	12/12	0.28 (12)	0.29 (12)	0.18 – 0.37
Pesticides				
Organophosphates (ppm):				
Malathion	6/12	0.03 (6)	0.04 (6)	0.02 – 0.08
Parathion	0/12	—	—	—
Diazinon	0/12	—	—	—
Chlorinated hydrocarbons (ppb):				
DDT	11/11	5 (11)	5 (11)	—
DDE	11/11	5 (11)	8 (2)	5 – 10
DDD	11/11	5 (11)	7 (2)	5 – 8
Aldrin	0/11	—	—	—
Dieldrin	11/11	5 (11)	5 (11)	—
Endrin	11/11	5 (11)	5 (11)	—
Heptachlor epoxide	11/11	5 (11)	5 (11)	—
Natural compounds (ppb)				
Aflatoxin B-1	0/12	—	—	—
B-2	0/12	—	—	—
G-1	0/12	—	—	—
G-2	0/12	—	—	—
Estrogen (ppb)	8/12	4 (8)	4 (8)	—
Miscellaneous				
Polychlorinated biphenyls (PCBs) (ppb)	11/12	10 (11)	20 (3)	10 – 37

in Table 4. The following contaminants were not detected in the cat diet: Penicillin, streptomycin, oxytetracycline, parathion, diazinon, the aflatoxins, and aldrin. Of the compounds detected, the following were measured in all feed samples: Metals and the chlorinated hydrocarbon pesticides (with the exception of aldrin). Chlortetracycline was found in 1 of 12 samples, malathion in 6 of 12 samples, estrogen in 8 of 12 samples, and PCBs in 11 of 12 samples. The concentrations of lead and manganese were found to be in the part per million range, with the levels of manganese being much higher than those of lead. The concentrations of other detected trace contaminants were in the part per billion range. The ranges of concentrations for detected contaminants were relatively narrow.

Monkey Diet

Purina Monkey Diet was collected and analyzed over a one-year period. The results of these analyses are presented in Table 5. The following were not found

Table 5. Results of analyses of monkey diet for trace contaminants

Substance	Samples detected / samples analyzed	Median (N)	Mean (N)	Range
Antibiotics (ppm)				
Penicillin	0/12	—	—	—
Streptomycin	0/12	—	—	—
Oxytetracycline	0/12	—	—	—
Chlortetracycline	0/12	—	—	—
Metals (ppm)				
Arsenic	12/12	0.12 (12)	0.13 (11)	0.07 – 0.21
Cadmium	12/12	0.09 (12)	0.10 (12)	0.07 – 0.15
Lead	12/12	0.11 (12)	0.13 (12)	0.098 – 0.21
Manganese	12/12	49.0 (12)	49.1 (12)	42.9 – 54.7
Mercury	12/12	<0.05 (12)	<0.05 (12)	—
Selenium	12/12	0.08 (12)	0.11 (11)	0.05 – 0.43
Pesticides				
Organophosphates (ppm):				
Malathion	2/12	—	0.04 (2)	0.04 – 0.04
Parathion	0/12	—	—	—
Diazinon	0/12	—	—	—
Chlorinated hydrocarbons (ppb):				
DDT	11/11	<5 (11)	<5 (11)	—
DDE	11/11	<5 (11)	<5 (11)	—
DDD	11/11	<5 (11)	<5 (11)	—
Aldrin	0/11	—	—	—
Dieldrin	11/11	<5 (11)	<5 (11)	—
Endrin	11/11	<5 (11)	<5 (11)	—
Heptachlor epoxide	11/11	<5 (11)	<5 (11)	—
Natural compounds (ppb)				
Aflatoxin B-1	0/12	—	—	—
B-2	0/12	—	—	—
G-1	0/12	—	—	—
G-2	0/12	—	—	—
Estrogen (ppb)	9/12	<4 (9)	<4 (9)	—
Miscellaneous				
Polychlorinated biphenyls (PCBs) (ppb)	11/12	<10 (11)	<10 (11)	—

in any samples of monkey feed: The antibiotics, parathion, diazinon, the aflatoxins, and aldrin. The trace metals and the chlorinated hydrocarbon insecticides (with the exception of aldrin) were found in all samples analyzed. Malathion was found in 2 of 12 samples, estrogen in 9 of 12 samples, and PCBs in 11 of 12 samples. With the exception of manganese, the measured levels of trace contaminants were in the parts per billion range. The ranges of values for measured concentrations were relatively narrow. As with other diets, the level of manganese was several orders of magnitude above those of other contaminants.

Rabbit Diet

Although samples of rabbit diet were collected and analyzed throughout a one-year period, the samples were collected at less frequent intervals than for

Table 6. Results of analyses of rabbit diet for trace contaminants

Substance	Samples detected / samples analyzed	Median (N)	Mean (N)	Range
Antibiotics (ppm)				
Penicillin	0/7	—	—	—
Streptomycin	0/7	—	—	—
Oxytetracycline	0/7	—	—	—
Chlortetracycline	0/7	—	—	—
Metals (ppm)				
Arsenic	7/7	0.18 (7)	0.20 (7)	0.14 – 0.42
Cadmium	7/7	0.11 (7)	0.12 (7)	0.10 – 0.15
Lead	7/7	0.50 (7)	0.47 (7)	0.34 – 0.57
Manganese	7/7	86.7 (7)	88.0 (7)	82.5 – 93.8
Mercury	7/7	<0.05 (7)	<0.05 (7)	—
Selenium	7/7	0.31 (7)	0.31 (7)	0.24 – 0.41
Pesticides				
Organophosphates (ppm)				
Malathion	7/7	0.05 (7)	0.06 (7)	0.04 – 0.09
Parathion	0/7	—	—	—
Diazinon	0/7	—	—	—
Chlorinated hydrocarbons (ppb)				
DDT	7/7	<5 (7)	<5 (7)	—
DDE	7/7	<5 (7)	<5 (7)	—
DDD	7/7	<5 (7)	<5 (7)	—
Aldrin	0/7	—	—	—
Dieldrin	7/7	<5 (7)	<5 (7)	—
Endrin	7/7	<5 (7)	<5 (7)	—
Heptachlor epoxide	7/7	<5 (7)	<5 (7)	—
Natural compounds (ppb)				
Aflatoxin B-1	0/7	—	—	—
B-2	0/7	—	—	—
G-1	0/7	—	—	—
G-2	0/7	—	—	—
Estrogen (ppb)	3/7	<4 (3)	<4 (3)	—
Miscellaneous				
Polychlorinated biphenyls (PCBs) (ppb)	7/7	<10 (7)	<10 (7)	—

the previously described diets. Consequently, only 7 samples were analyzed throughout a 12-month period. The results of these analyses are presented in Table 6. The antibiotics, parathion, diazinon, the aflatoxins, and aldrin were not found in any of these samples. By contrast, the metals, PCBs, malathion, and the chlorinated hydrocarbon insecticides (with the exception of aldrin) were measured in all samples collected. Estrogen was found in only 3 of 7 samples. With the exception of managanese, the levels of trace contaminants were in the part per billion range. Manganese was several orders of magnitude higher in concentration than that of other trace contaminants. The range of concentrations for all contaminants analyzed was relatively narrow.

Hamster Diet

Because of the relatively limited amount of toxicity work with hamsters in our laboratory, hamster diet was available on only two occasions throughout the

Table 7. Results of analyses of hamster diet for trace contaminants

Substance	Samples detected / samples analyzed	Median (N)	Mean (N)	Range
Antibiotics (ppm)				
Penicillin	0/2	—	—	—
Streptomycin	0/2	—	—	—
Oxytetracycline	0/2	—	—	—
Chlortetracycline	0/2	—	—	—
Metals (ppm)				
Arsenic	2/2	—	0.17 (2)	0.10 – 0.24
Cadmium	2/2	0.12 (2)	0.12 (2)	—
Lead	2/2	—	1.07 (2)	0.35 – 1.79
Manganese	2/2	—	82.6 (2)	81.6 – 83.5
Mercury	2/2	<0.05 (2)	<0.05 (7)	—
Selenium	2/2	—	0.34 (2)	0.25 – 0.42
Pesticides				
Organophosphates (ppm)				
Malathion	2/2	—	0.05 (2)	0.04 – 0.06
Parathion	0/2	—	—	—
Diazinon	0/2	—	—	—
Chlorinated hydrocarbons (ppb)				
DDT	2/2	<5 (2)	<5 (2)	—
DDE	2/2	<5 (2)	<5 (2)	—
DDD	2/2	<5 (2)	<5 (2)	—
Aldrin	0/2	—	—	—
Dieldrin	2/2	<5 (2)	<5 (2)	—
Endrin	2/2	<5 (2)	<5 (2)	—
Heptachlor epoxide	2/2	<5 (2)	<5 (2)	—
Natural compounds (ppb)				
Aflatoxin B-1	0/2	—	—	—
B-2	0/2	—	—	—
G-1	0/2	—	—	—
G-2	0/2	—	—	—
Estrogen (ppb)	2/2	<4 (2)	—	—
Miscellaneous				
Polychlorinated biphenyls (PCBs) (ppb)	2/2	<10 (2)	<10 (2)	—

sampling period. Samples of each of these batches of hamster diet were subjected to analysis for trace contaminants. The results are presented in Table 7. Not detected in either sample were the antibiotics, parathion, diazinon, the aflatoxins, and aldrin. The following contaminants were found in both samples: Metals, estrogen, PCBs, and chlorinated hydrocarbon insecticide (with the exception of aldrin). Both lead and manganese were measured in the parts per million range; whereas, the levels of other trace contaminants was consistently in the part per billion range.

Discussion and Conclusions

Some observations were consistent regardless of which diet was analyzed or when the samples were collected from specific diets. The antibiotics, two of the organophosphates (parathion and diazinon) and the aflatoxins (B_1, B_2, G_1, and

G_3) were uniformly absent from the diets. By contrast, trace elements such as arsenic, cadmium, lead, manganese, mercury and selenium were consistently present, as were the PCBs and the chlorinated hydrocarbon insecticides (with the exception of aldrin). The importance of knowing the concentration of PCBs is related not to their innate toxicity, but to their ability to alter the metabolic rates of other foreign compounds. Since toxicologic studies in which these diets are used are aimed at identifying the toxicity of individual study compounds, it is important that agents, which would interfere with the accuracy of toxicity measurements, be identified. Thus the toxicity of the PCBs is unimportant in this context.

It is left up to the investigator to determine whether specific levels of PCBs warrant further analysis with respect to the identification of individual components. The analysis of PCB mixtures thus serves as a useful screen to determine agents that may possibly alter the outcome of experiments through the modification of responses to the study agent. Estrogen and malathion were observed on a less frequent basis. The estrogen assay, as conducted, is a pass or fail type and as designed cannot measure a specific level of activity. It tells the investigator whether the diet has less than or more than four ppb of estrogenic activity as measured against diethylstilbesterol. It has been observed that "estrogenic-like" activity is not always animal related. Plant materials can cause the same hormonal effect from phytosterol and cause phytoesterogenic activity. This is quite common in swine feeds, particularly with corn which is old or has some mold growth. Manganese was present in the highest concentrations measured and was consistently present in the part per million range. Lead was found occasionally in the part per million range; however, it was observed more frequently in the part per billion range. All other trace contaminants that were identified in the animal feeds were in the part per billion range. There appeared to be no cyclic change in concentration for the detected trace contaminants. Of those contaminants present in feeds, the levels of occurrence fell within relatively narrowly defined ranges. For the chlorinated hydrocarbon insecticides, the levels were too low to quantify, despite the constant occurrence of these contaminants.

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